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Inhibition of Flesh Browning and Skin Color Fading in Frozen Fillets of Yelloweye Snapper (*Lutjanus vivanus*)

HAROLD C. THOMPSON, JR. and MARY H. THOMPSON

ITTLE, WA
ruary 1972

National Marine Fisheries Service, Special Scientific Report--Fisheries Series

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Inhibition of Flesh Browning and Skin Color Fading in Frozen Fillets of Yelloweye Snapper (*Lutjanus vivanus*)

By

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ABSTRACT

Fresh yelloweye snapper (*Lutjanus vivanus*) which had been scaled and eviscerated were treated with the chemicals 3,3′-thiodipropionic acid, glutathione, disodium ethylenediaminetetraacetate dihydrate in combination with propyl gallate, and monotertiary butylhydroquinone. The inhibitory effects that these chemicals had on flesh browning (Maillard reaction) were studied over a 12-month frozen storage period. Also, the effects of vacuum packaging on snapper skin discoloration were studied.

INTRODUCTION

The Gulf of Mexico red snapper fishery originated off the northwest Florida coast 15 to 20 years prior to the Civil War (Carpenter, 1965). Catches at that time were made with the use of handlines from sailing vessels containing live wells. At that time, ice for use on commercial vessels was too expensive owing to cost of shipping from the point of manufacture.

The snapper fishery did not start on a large scale until after the end of the Civil War (Warren, 1898). The fishery really began to bloom when ice manufacturing plants began producing ice at a reasonable cost. With ice to cool their catches, the crews of the snapper vessels were able to go out farther and stay longer. Another boost was given to the fishery around 1920 when sail-rigged vessels became diesel powered. The majority of the snapper fleet still ices the fish in the hold; however, a few new freezer vessels have entered the fleet.

The total landings of snapper in the United States for 1968 were 11,500,000 lb. valued at $3,700,000 while the totals for 1969 were 9,500,000 lb. valued at $4,000,000 (Riley, 1970).

The present methods used in commercial processing of snapper are limited to (1) evisceration and freezing the whole fish and (2) filleting or steaking the fish and then freezing them. The majority of the snapper are either sold in a fresh iced condition or as eviscerated frozen fish. The flesh of the fillets and steaks turns from white to brown in color during extended periods of frozen storage, thus limiting the market owing to lack of consumer eye appeal. The red skin on the fillets and steaks, one of the snapper's main selling points, also fades in color to a beige or bronze during frozen storage. Skin coloration is used by most large firms as a quality indicator.

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There are three broad types of browning reactions recognized in foods. The first is caramelization which occurs when polyhydroxycarbonyl compounds are heated to relatively high temperatures in the absence of amino acids. The second is a group of oxidative reactions which convert ascorbic acid and polyphenols into di- or polycarbonyl compounds. The third type, which is the most common, involves the reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, and proteins and is known as the Maillard reaction.

Liston *et al.* (1963) state that the free amino groups of fish proteins are available for reactions with reducing sugars or with some of the products of oxidation of lipid material. The end result of a complicated series of reactions is the formation of brown colors as well as changes in flavor.

According to Von Tigerstrom and Tarr (1965), the browning which occurs in muscles of many species of fish post mortem is due to the Maillard reaction. Von Tigerstrom and Tarr (1965) also brought out the importance of D-ribose as playing a significant role in the Maillard reaction. Other researchers have also indicated that ribose is one of the reducing sugars which is a main contributor to the Maillard reaction (Tarr and Gadd, 1965; Pomeranz *et al.*, 1962; Gilka, 1963; and Jones, 1962).

Most browning inhibitors have been shown to be carbonyl reagents such as cyanide (Barnes and Kaufman, 1947), hydroxylamine (Burton, 1945), hydrazine (Barnes and Kaufman, 1947), mercaptans (Guss, 1952; Song and Chichester, 1967), bisulfite (Olcott, 1953), and glutathione (Joslyn and Ponting, 1951). Most of these chemicals, however, have undesirable side effects in meat and fish. Other methods used in inhibiting the flesh browning are (1) low storage temperatures and (2) lowering the moisture content in dehydrated foods.

In spite of the commercial importance of good skin color of red snapper, there has been very little work done on preventing skin color fading. According to Tsukuda and Amano (1966), storage temperatures as low as —30° C did not prevent skin discoloration in *Lepidotrigla güntheri.* Lusk *et al.* (1964) reported the changes in the amount of astacene pigment in freeze-dried shrimp during freeze dehydration and subsequent storage both in air atmosphere and a nitrogen atmosphere. The pigment in shrimp stored in the air atmosphere was essentially all bleached after one week whereas shrimp stored in a nitrogen atmosphere retained their pigment color over the same period. Yamaga and Morioka (1962) described the effectiveness of certain antioxidants used to prevent the skin color fading in certain red fishes.

The purposes of this paper are to describe an experiment designed to show (1) the inhibitory effects that certain chemicals have on the browning of the flesh of frozen snapper fillets and (2) the effect vacuum packaging has on preventing the skin discoloration of frozen snapper fillets.

MATERIALS AND METHODS

Preparation of Samples

Yelloweye snapper *(Lutjanus vivanus).* — Fifty-two fresh, iced, eviscerated, heads-on snapper were obtained from a local fishery. These fish were scaled and headed. Their weights averaged approximately 3 lb.

Chemicals.—All chemicals were either food grade or reagent grade quality. The chemicals used in this study were 3,3′-thiodipropionic acid (TDP), glutathione (Glu), disodium ethylenediaminetetraacetate dihydrate (Na_2EDTA) in combination with propyl gallate (PG), and monotertiary butylhydroquinone (TBHQ).

Method of chemical application. — The technique involved using a hypodermic syringe to inject a scaled eviscerated snapper prior to filleting with an aqueous solution of the desired chemical. Each side of the fish was injected with two 2-ml shots evenly spaced down the lateral line. The solutions of TDP and glutathione were injected in the amount necessary to produce a residual concentration of the chemicals equivalent to 0.02% of the snapper oil content. TBHQ was injected in an amount to produce a 50 ppm residual. A combination of Na_2EDTA and PG was injected in the amount to produce a 50 ppm residual of the combina-

tion. These chemicals can also be applied to fillets by a dipping or injection technique as was demonstrated by this laboratory (Thompson and Thompson, 1969).

Packaging. — All fillets were separately packaged in vacuum-sealed Cryovac plastic bags.

Storage. — All fillets were stored in a —10° F (—23.3° C) freezer until analyzed.

Organoleptic Evaluation of Fillets

Fillets were evaluated organoleptically by an 8-member expert taste panel at the end of 1, 3, 6, 9, and 12 months of frozen storage. The fillets were scored on appearance, taste, and texture. The scoring method consisted of giving a score of from 5 for excellent to 0 for inedible.

Chemical Methods

Total ribose determination. — Total ribose was extracted from minced snapper flesh according to the method of Sinnhuber (1966). Total ribose content of the extract was determined by the orcinol method of Mejbum (1939). Heating time was increased to 45 min as modified by Albaum and Umbreit (1947).

Free ribose determination. — Free ribose was extracted from minced snapper flesh according to the method of Sinnhuber (1966). Bound ribose was removed from this extract using Dowex ion exchange resins according to the method of Tarr and Leroux (1962). Free ribose was then determined by the orcinol method of Mejbum (1939) using an increased heating time of 45 min as modified by Albaum and Umbreit (1947).

RESULTS

Organoleptically the glutathione treated fillets received the best average score (4.7) at the end of 1 month of storage (Table 1). By the end of 3 months of storage, the control, glutathione, and the Na_2EDTA + PG treated fillets all received an average score of 4.0. Not until the 6 month draw was there a considerable difference in organoleptic scores observed. Then both the TDP and glutathione treated fillets received a score of 3.8 while the control fillets received a score of 3.2. By the end of 12 months of storage the TDP treated fillets received the highest score (2.7) while the NA_2EDTA + PG treated fillets received the lowest score (1.8). After averaging scores for appearance only (Table 2), the TDP treated fillets received the

Table 1.--Organoleptic scores for cooked snapper fillets stored at -10° F.[1/]

Sample	Storage period				
	1 month	3 months	6 months	9 months	12 months
Control	4.6	4.0	3.2	2.0	2.4
TDP	--	--	3.8	3.5	2.7
Glu	4.7	4.0	3.8	2.9	2.2
Na_2EDTA + PG	4.5	4.0	3.3	3.6	1.8
TBHQ	--	--	3.0	2.9	2.2

[1/]Values presented are averages of scores given for appearance, taste, and texture based on a 5-point scale.

Table 2.--Appearance scores for cooked snapper fillets stored at -10° F.[1/]

Sample	Storage period				
	1 month	3 months	6 months	9 months	12 months
Control	4.8	4.0	4.2	2.2	2.6
TDP	--	--	5.0	3.7	2.7
Glu	4.4	4.0	5.0	2.9	2.4
Na_2EDTA + PG	4.6	4.0	4.2	3.6	1.8
TBHQ	--	--	4.2	3.4	2.2

[1/]Values presented are averages of scores given for appearance by taste panel members based on a 5-point scale.

Table 3.--Free and total ribose content of snapper flesh stored at -10° F.[1/]

Sample	Storage period					
	1 month	2 months	3 months	6 months	9 months	12 months
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Free ribose						
Control	13.64	12.93	13.24	11.14	10.43	9.71
TDP	10.87	10.33	11.01	5.19	4.78	8.57
Glu	11.10	10.47	7.61	10.27	10.44	10.71
Na_2EDTA + PG	12.15	4.18	7.34	5.07	4.78	7.33
TBHQ	19.27	8.01	16.29	3.11	2/	6.88
Total ribose						
Control	31.15	21.51	26.21	21.16	19.88	15.77
TDP	39.15	18.27	35.82	27.39	21.51	35.19
Glu	33.54	19.78	23.84	14.32	28.05	24.91
Na_2EDTA + PG	36.88	25.24	28.24	20.01	35.72	23.91
TBHQ	38.04	17.21	28.72	24.43	17.35	25.70

[1/]Values presented are averages of duplicate samples.
[2/]Sample was lost.

highest score (2.7) at the end of 12 months of frozen storage while the Na_2EDTA + PG treated fillets were given the lowest score for appearance (1.8) over the same storage period.

Visual observations of the progression of browning are supported by changes in free and total ribose content of snapper flesh over a 12-month frozen storage period. The greatest decrease in free ribose content of snapper fillets between 1 and 12 months of frozen storage was exhibited by fillets treated with TBHQ, NA_2-EDTA + PG, and by the control (Table 3). The fillets treated with TDP and glutathione showed the least differences in free ribose content over the same period. The fillets that showed the largest decreases in total ribose content over the 12-month storage period were the control, those treated with Na_2EDTA + PG, and those treated with TBHQ. The least change in total ribose content over the 12-month storage period was observed for the TDP and glutathione treated fillets.

By the end of 3 months of frozen storage, the flesh of the control, the Na_2EDTA + PG, and the TBHQ treated fillets were noticeably turning brown. By the end of 6 months of storage, these fillets had turned even browner, approaching the color of a brown pasteboard box. The flesh of the TDP and glutathione treated fillets, however, was nearly as white as that of a freshly filleted snapper. The TDP treated fillets received the highest organoleptic score for appearance throughout the storage period. Between 9 and 12 months of frozen storage, the texture of all the fillets became woody or slightly tough.

The skin color deterioration problem was successfully met by packaging the fillets in vacuum-sealed Cryovac bags. None of the samples deteriorated appreciably with regard to skin color over the 12-month frozen storage period. There was no difference noted in skin color between the control fillets or the fillets treated with the chemicals used in this experiment.

DISCUSSION

The organoleptic scores for both TDP and TBHQ treated fillets at the 1- and 3-month draws were not obtained since both of these chemicals were still considered experimental. Subsequently, we learned that both chemicals were approved for use in foods by the Food and Drug Administration (FDA) and organoleptic studies were initiated on the fillets treated with these chemicals.

Bound ribose is present in fish flesh in the form of nucleic acids such as RNA, nucleotides such as IMP, and nucleosides such as inosine, to name a few. The majority of the free ribose present in fish flesh is produced by the post mortem degradation of one or more of the general classes of compounds mentioned above. Several researchers (Von Tigerstrom and Tarr, 1965; Tarr and Gadd, 1965; and Pomeranz *et al.*, 1962) have indicated that it is the free ribose which is involved in the Maillard reaction in post mortem browning of fish muscle.

The test pack of red snapper was stored in a —10° F (—23.3° C) freezer. At the slightly warmer storage temperature of —18° C, the nucleotide IMP (inosine monophosphate) in frozen swordfish steaks is degraded at the rate of only 0.029 μmoles per gram per week. IMP degrades at the rate of 0.24 μmoles per gram per week at —8° C (Dyer and Hiltz, 1969). This type of degradation results in a change in the free and bound ribose content of fish flesh. According to Dyer *et al.* (1966), no significant change in content of IMP, inosine, and hypoxanthine in the ordinary muscle of fast-frozen swordfish steaks occurs during freezing and frozen storage at —26° C for a period of from 4 to 5 months. Therefore, at the temperature at which this pack was stored (approximately —26° C), no changes in free and bound ribose due to enzymatic degradation of nucleotides and nucleosides should have occurred. Consequently, the progress of browning in the frozen snapper flesh can be followed by analyzing the flesh for free and total ribose.

As browning progresses in frozen snapper flesh, the amount of free ribose decreases due to a reaction between ribose and a free amino acid or the amino group of a protein; this reaction is the first of a series of complicated reactions which occur in the browning process.

The greatest decrease in free ribose content occurred in the control fillets and those which had been treated with TBHQ and Na_2EDTA + PG. The least amount of decrease in free ribose content occurred in those fillets which had been treated with TDP and glutathione.

These free ribose data parallel the visual observations of the progression of the browning of the snapper flesh. The flesh of the TDP treated fillets, when visually observed, was consistently lighter in color at each draw than the flesh of any of the other fillets.

Taking into account the ribose data, organoleptic scores, and visual observations of the progression of browning, the TDP treated fillets showed a much better inhibition of browning, as well as preservation of overall quality, than fillets treated with other chemicals. The foregoing combination of observations indicates that browning in snapper flesh is that of the Maillard type.

The skin color deterioration of frozen snapper fillets is an oxidative process whereby the red skin pigment of snapper, astaxanthin, is converted to astacene or further degradation products. The red pigment in marine fishes is predominantly astaxanthin (Tsukuda and Amano, 1966). In an earlier experiment conducted at this laboratory, several of the more common antioxidants used in the food industry were applied to snapper fillets (Love and Thompson, 1969). Application of these antioxidants deters the fading of the skin color for only a few months. In the present experiment, however, packaging the fillets in Cryovac vacuum-sealed plastic bags inhibited the oxidative process sufficiently to increase the frozen storage shelf life to 12 months. After this period, a slight discoloration or fading of the red pigment was noted. This indicated that the oxidative process which converts the red pigment, astaxanthin, to astacene or products further down the degradation chain is not entirely due to the presence of air. Enzymatic oxidation also probably plays an important role in the skin color deterioration of red snapper.

The application of TDP to red snapper fillets followed by Cryovac vacuum packaging and subsequent freezing of the product results in a superior quality product for a 12-month storage period. The TDP can be applied by either dipping or injecting the fillets as was shown by previous experiments at this laboratory (Thompson and Thompson, 1969). Either method of application produces the same results. This treatment is by no means a "cure-all" for the snapper industry, but it is a beginning since the previous storage life of a frozen snapper fillet was somewhere between 1 and 3 months.

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